

WE CLAIM:

- 5 1. A genomic DNA containing a BRCA2 gene,
 wherein the first twelve nucleotides beginning exon 5 are 5'-
 TCCTGTTGTTCT-3' as set forth in SEQ. ID. NO: 1,
 wherein nucleotides numbers 5782-5790 are GTTTGTGTT as set forth in
 SEQ. ID. NO: 4, and
10 wherein the last 20 nucleotides ending exon 15 are 5'-
 CTGCGTGTTCTCATAAACAG-3' as set forth in SEQ. ID. NO: 2 and the first 20
 nucleotides beginning exon 16 are 5'-CTGTATACGTATGGCGTTTC-3' as set forth
 in SEQ. ID. NO: 3.
- 15 2. The genomic DNA according to claim 1 wherein the coding sequence
 nucleotides are as follows:
20 1093 A
 1342 A
 1593 A
 2457 T
 2908 G
 3199 A
 3624 A
 4035 T
25 7470 A
 9079 G.
- 30 3. The genomic DNA according to claim 1 wherein the coding sequence
 nucleotides are as follows:
35 1093 A
 1342 C
 1593 A
 2457 T
 2908 G
 3199 A
 3624 A
 4035 T
40 7470 A
 9079 G.
- 45 4. The genomic DNA according to claim 1 wherein the coding sequence
 nucleotides are as follows:

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	1093 A
	1342 C
5	1593 A
	2457 T
	2908 G
	3199 A
	3624 A
10	4035 C
	7470 A
	9079 G.

15 5. The genomic DNA according to claim 1 wherein the coding sequence
nucleotides are as follows:

	1093 C
	1342 A
20	1593 A
	2457 C
	2908 G
	3199 G
	3624 G
25	4035 T
	7470 G
	9079 G.

6. The genomic DNA according to claim 1 wherein the coding sequence
30 nucleotides are as follows:

	1093 A
	1342 C
	1593 A
35	2457 T
	2908 G
	3199 A
	3624 G
	4035 T
40	7470 G
	9079 G.

7. The genomic DNA according to claim 1 wherein the coding sequence nucleotides are as follows:

x	y
45	1093 C
50	2457 C
1093 C	45
1342 C	50
1593 G	
2457 C	
2908 A	
3199 G	

3624 A
4035 T
7470 A
9079 A.

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8. The genomic DNA according to claim 1 wherein the coding sequence nucleotides are as follows:

10 2024 C
4553 C
4815 G
5841 T
5972 C.

15 9. A DNA comprising a BRCA2 coding sequence, wherein nucleotide numbers 643-666 are CTTAGTGAAAGTCCTGTTGTTCTA and wherein nucleotides numbers 5782-5790 are GTTTGTGTT.

20 10. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

25 1093 A
1342 A
1593 A
2457 T
2908 G
3199 A
30 3624 A
4035 T
7470 A
9079 G.

35 11. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

40 1093 A
1342 C
1593 A
2457 T
2908 G
3199 A
45 3624 A
4035 T
7470 A
9079 G

as set forth in SEQ. ID. NO: 4.

50 12. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

1093 A
1342 C
1593 A
2457 T
2908 G
3199 A
3624 A
4035 C
7470 A
9079 G

5 10 as set forth in SEQ. ID. NO: 6.

15 13. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

1093 C
1342 A
1593 A
2457 C
2908 G
3199 G
3624 G
4035 T
7470 G
9079 G

20 25 30 as set forth in SEQ. ID. NO: 8.

35 14. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

1093 A
1342 C
1593 A
2457 T
2908 G
3199 A
3624 G
4035 T
7470 G
9079 G

40 45 50 as set forth in SEQ. ID. NO: 10.

45 15. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

1093 C
1342 C
1593 G
2457 C

2908 A
3199 G
3624 A
4035 T
7470 A
9079 A

as set forth in SEQ. ID. NO: 12.

16. The DNA according to claim 9 wherein the coding sequence nucleotides are as follows:

2024 C
4553 C
4815 G
5841 T
5972 C.

17. A BRCA2 protein having the following amino acids at the following peptide numbers:

289 asparagine
372 histidine
894 valine
991 asparagine
1852 valine
1853 cysteine
1854 valine
2951 alanine

as set forth in SEQ. ID. NO: 5.

18. The BRCA2 protein having the following amino acids at the following peptide numbers:

289 asparagine
372 asparagine
599 serine
894 valine
991 asparagine
2951 alanine.

19. The BRCA2 protein having the following amino acids at the following peptide numbers:

289 histidine
372 histidine
894 valine
991 aspartic acid
2951 alanine

as set forth in SEQ. ID. NO: 9.

20. The BRCA2 protein having the following amino acids at the following peptide numbers:

5 289 histidine
372 asparagine
894 isoleucine
991 aspartic acid
2951 threonine

10 as set forth in SEQ. ID. NO: 13.

21. The BRCA2 protein according to claims 17-20 having the following amino acids at the following peptide numbers:

15 599 serine
1442 serine
1915 threonine.

22. A haplotype of BRCA2 coding sequence (BRCA2^{omi 1}) as set forth in SEQ. ID. NO: 4 or a sequence complementary thereto.

23. A BRCA2 protein comprising an amino acid sequence derived from BRCA2^{omi 1} as set forth in SEQ. ID. NO: 5.

25 24. A haplotype of BRCA2 coding sequence (BRCA2^{omi 2}) as set forth in SEQ. ID. NO: 6 or a sequence complementary thereto.

25. A BRCA2 protein comprising an amino acid sequence derived from BRCA2^{omi 2} as set forth in SEQ. ID. NO: 7.

30 26. A haplotype of BRCA2 coding sequence (BRCA2^{omi 3}) as set forth in SEQ. ID. NO: 8 or a sequence complementary thereto.

27. A BRCA2 protein comprising an amino acid sequence derived from BRCA2^{omi 3} as set forth in SEQ. ID. NO: 9.

28. A haplotype of BRCA2 coding sequence (BRCA2^{omi}⁴) as set forth in SEQ. ID.
NO: 10 or a sequence complementary thereto.
- 5 29. A BRCA2 protein comprising an amino acid sequence derived from BRCA2^{omi}⁴ as set forth in SEQ. ID. NO: 11.
- 10 30. A haplotype of BRCA2 coding sequence (BRCA2^{omi}⁵) as set forth in SEQ. ID.
NO: 12 or a sequence complementary thereto.
- 15 31. A BRCA2 protein comprising an amino acid sequence derived from BRCA2^{omi}⁵ as set forth in SEQ. ID. NO: 13.
- 20 32. A method of identifying individuals having a BRCA2 gene with a BRCA2 coding sequence not associated with disease, comprising:
- (a) amplifying a DNA or a fragment thereof of an individual's BRCA2 coding sequence;
- (b) sequencing said amplified DNA fragment;
- (c) if necessary, repeating steps (a) and (b) until said individual's BRCA2 coding sequence is sufficiently sequenced to determine whether a mutation is present;
- (d) comparing the sequence of said amplified DNA fragment to a BRCA2^(omi) DNA sequence selecting from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences;
- (e) determining the presence of absence of each of the following polymorphic variations in said individual's BRCA2 coding sequence:
- (i) AAT and CAT at position 1093,
- (ii) CAT and AAT at position 1342,
- (iii) TCA and TCG at position 1593,

- (iv) CAT and CAC at position 2457,
(v) GTA and ATA at position 2908,
(vi) AAC and GAC at position 3199,
5 (vii) AAA and AAG at position 3624,
(viii) GTT and GTC at position 4035,
(ix) TCA and TCG at position 7470, and
(x) GCC and ACC at position 9079; and
- (f) determining any sequence differences between said individual's BRCA2 coding sequences and a BRCA2^(omni) DNA sequence selected from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences, wherein the presence of said polymorphic variations and the absence of a variation outside of positions 1093, 1342, 1593, 2457, 2908, 3199, 3624, 4035, 7470, and 9079 is correlated with an absence of increased genetic susceptibility to breast or ovarian cancer resulting from a BRCA2 mutation in the BRCA2 coding sequence.
33. A method of identifying individuals having a BRCA2 gene with a BRCA2 coding sequence not associated with disease, comprising:
- 20 (a) amplifying a DNA or a fragment thereof of an individual's BRCA2 coding sequence;
(b) sequencing said amplified DNA fragment;
(c) if necessary, repeating steps (a) and (b) until said individual's BRCA2 coding sequence is sufficiently sequenced to determine whether a mutation is present;
- 25 (d) comparing the sequence of said amplified DNA fragment to a BRCA2^(omni) DNA sequence selecting from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences;

(e) determining the presence or absence of each of the following polymorphic variations in said individual's BRCA2 coding sequence:

- (i) AAT and CAT at position 1093,
- (ii) CAT and AAT at position 1342,
- (iii) TCA and TCG at position 1593,
- (iv) CAT and CAC at position 2457,
- (v) GTA and ATA at position 2908,
- (vi) AAC and GAC at position 3199,
- (vii) AAA and AAG at position 3624,
- (viii) GTT and GTC at position 4035,
- (ix) TCA and TCG at position 7470, and
- (x) GCC and ACC at position 9079; and

(f) determining any sequence differences between said individual's BRCA2 coding sequences and a BRCA2^(om) DNA sequence selected from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences, wherein the presence of said polymorphic variations and the absence of a variation outside of positions 1093, 1342, 1593, 2457, 2908, 3199, 3624, 4035, 7470, and 9079 is correlated with an absence of increased genetic susceptibility to breast or ovarian cancer resulting from a BRCA2 mutation in the BRCA2 coding sequence; wherein, codon variations occur at the following frequencies, respectively, in a Caucasian population of individuals free of disease:

- (i) at position 1093, AAT and CAT occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (ii) at position 1342, CAT and AAT occur at frequencies from about 35-45%, and from about 55-65%, respectively,
- (iii) at position 1593, TCA and TCG occur at frequencies from about 85-95%, and from about 5-15%, respectively,

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- (iv) at position 2457, CAT and CAC occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (v) at position 2908, GTA and ATA occur at frequencies from about 85-95%, and from about 5-15%, respectively,
- (vi) at position 3199, AAC and GAC occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (vii) at position 3624, AAA and AAG occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (viii) at position 4035, GTT and GTC occur at frequencies from about 85-95%, and from about 5-15%, respectively,
- (ix) at position 7470, TCA and TCG occur at frequencies from about 75-85%, and from about 15-25%, respectively, and
- (x) at position 9079, GCC and ACC occur at frequencies from about 85-95%, and from about 5-15%, respectively.

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34. A method of detecting an increased genetic susceptibility to breast and
20 ovarian cancer in an individual resulting from the presence of a mutation in the
BRCA2 coding sequence, comprising:

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- (a) amplifying a DNA or a fragment thereof of an individual's BRCA2 coding sequence;
- (b) sequencing said amplified DNA fragment;
- (c) if necessary, repeating steps (a) and (b) until said individual's BRCA2 coding sequence is sufficiently sequenced to determine whether a mutation is present;
- (d) comparing the sequence of said amplified DNA fragment to a BRCA2^(oml) DNA sequence selected from the group consisting of SEQ.

(e) determining any sequence differences between said individual's BRCA2 coding sequences and a BRCA2^(om) DNA sequence selected from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences;

(i) AAT and CAT at position 1093,
(ii) CAT and AAT at position 1342,
(iii) TCA and TCG at position 1593,
(iv) CAT and CAC at position 2457,
(v) GTA and ATA at position 2908,
(vi) AAC and GAC at position 3199,
(vii) AAA and AAG at position 3624,
(viii) GTT and GTC at position 4035,
(ix) TCA and TCG at position 7470, and
(x) GCC and ACC at position 9079, is correlated with the potential of increased genetic susceptibility to breast or ovarian cancer resulting from a BRCA2 mutation in the BRCA2 coding sequence.

35. A method of detecting an increased genetic susceptibility to breast and ovarian cancer in an individual resulting from the presence of a mutation in the BRCA2 coding sequence, comprising:

(a) amplifying a DNA or a fragment thereof of an individual's BRCA2 coding sequence;
(b) sequencing said amplified DNA fragment;

- (c) if necessary, repeating steps (a) and (b) until said individual's BRCA2 coding sequence is sufficiently sequenced to determine whether a mutation is present;
- 5 (d) comparing the sequence of said amplified DNA fragment to a BRCA2^(oml) DNA sequence selected from the group consisting of: SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences;
- 10 (e) determining any sequence differences between said individual's BRCA2 coding sequences and a BRCA2^(oml) DNA sequence selected from the group consisting of: SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, and their respective complementary sequences in order to determine the presence or absence of base changes in said individual's BRCA2 coding sequence wherein a base change which is not any one of the following:
- 15 (i) AAT and CAT at position 1093,
(ii) CAT and AAT at position 1342,
(iii) TCA and TCG at position 1593,
(iv) CAT and CAC at position 2457,
20 (v) GTA and ATA at position 2908,
(vi) AAC and GAC at position 3199,
(vii) AAA and AAG at position 3624,
(viii) GTT and GTC at position 4035,
(ix) TCA and TCG at position 7470, and
25 (x) GCC and ACC at position 9079, is correlated with the potential of increased genetic susceptibility to breast or ovarian cancer resulting from a BRCA2 mutation in the BRCA2 coding sequence, wherein, codon variations occur at the following frequencies, respectively, in a Caucasian population of individuals free of disease:

- (i) at position 1093, AAT and CAT occur at frequencies from about 75-85%, and from about 15-25%, respectively,
5 (ii) at position 1342, CAT and AAT occur at frequencies from about 35-45%, and from about 55-65%, respectively,
10 (iii) at position 1593, TCA and TCG occur at frequencies from about 85-95%, and from about 5-15%, respectively,
15 (iv) at position 2457, CAT and CAC occur at frequencies from about 75-85%, and from about 15-25%, respectively,
20 (v) at position 2908, GTA and ATA occur at frequencies from about 85-95%, and from about 5-15%, respectively,
25 (vi) at position 3199, AAC and GAC occur at frequencies from about 75-85%, and from about 15-25%,
respectively,
(vii) at position 3624, AAA and AAG occur at frequencies from about 75-85%, and from about 15-25%,
respectively,
(viii) at position 4035, GTT and GTC occur at frequencies from about 85-95%, and from about 5-15%, respectively,
(ix) at position 7470, TCA and TCG occur at frequencies from about 75-85%, and from about 15-25%, respectively, and
(x) at position 9079, GCC and ACC occur at frequencies from about 85-95%, and from about 5-15%, respectively.
36. A method according to any of the claims 32-35 wherein the said amplifying is performed by annealing at least one oligonucleotide primer to said DNA fragment and extending the oligonucleotide primer by an agent for polymerization.

37. A method according to claim 36 wherein said oligonucleotide primer is directly or indirectly labeled with a radioactive label, a fluorescent label, a bioluminescent label, a chemiluminescent label, a metal chelator, or an enzyme label.

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38. A BRCA2 coding sequence according to claims 32, wherein the codon pairs occur at the following frequencies:

- (i) at position 1093, AAT and CAT occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (ii) at position 1342, CAT and AAT occur at frequencies from about 35-45%, and from about 55-65%, respectively,
- (iii) at position 1593, TCA and TCG occur at frequencies from about 85-95%, and from about 5-15%, respectively,
- (iv) at position 2457, CAT and CAC occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (v) at position 2908, GTA and ATA occur at frequencies from about 85-95%, and from about 5-15%, respectively,
- (vi) at position 3199, AAC and GAC occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (vii) at position 3624, AAA and AAG occur at frequencies from about 75-85%, and from about 15-25%, respectively,
- (viii) at position 4035, GTT and GTC occur at frequencies from about 85-95%, and from about 5-15%, respectively,
- (ix) at position 7470, TCA and TCG occur at frequencies from about 75-85%, and from about 15-25%, respectively, and
- (x) at position 9079, GCC and ACC occur at frequencies from about 85-95%, and from about 5-15%, respectively.

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39. An oligonucleotide primer capable of hybridizing to a sample of BRCA2 gene, or its respective complementary sequences selected from the group consisting of SEQ. ID. NO: 14, 19, 22, 23, 25, 26, 29-76, 83, 85-88, 90, 91, 97, 98, 101, and 104-
5 107.
40. A chip array having "n" elements for performing allele specific sequence-based techniques comprising a solid phase chip and oligonucleotides having "n" different nucleotide sequences,
10 wherein "n" is an interger greater than or equal to ten,
wherein said oligonucleotides are bound to said solid phase chip in a manner which permits said oligonucleotides to effectively hybridize to complementary oligonucleotides or polynucleotides,
15 wherein oligonucleotides having different nucleotide sequence are bound to said solid phase chip at different locations so that a particular location on said solid phase chip exclusively binds oligonucleotides having a specific nucleotide sequence, and
20 wherein at least ten oligonucleotides are capable of specifically hybridizing to the BRCA2 DNA having the sequence as set forth in SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12 or their respective complementary sequences, at least one oligonucleotide being capable of specifically hybridizing at each of the nucleotide positions 1093, 1342, 1593, 2457, 2908, 3199, 3624, 4035, 7470, 9079, or complementary thereto.
25 41. A method of performing gene therapy on a patient, comprising:
a) contacting cancer cells *in vivo* with an effective amount of a vector comprising DNA containing at least a portion of BRCA2 sequence selected from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, or their respective complementary sequences
30 b) allowing the vector to enter the cancer cells, and
c) measuring a reduction in tumor growth.
42. The method according to claim 41 wherein said cancer cells have a mutation in the BRCA2 gene.

43. The method according to claim 41 wherein said patient has a mutation in the BRCA2 gene in non-cancer cells.

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44. A method of performing gene therapy on a patient or a sample, comprising:

a) contacting cells *in vivo* or *in vitro* with an effective amount of a vector comprising DNA containing at least a portion of BRCA2 sequence selected from the group consisting of SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO:

10 10, SEQ. ID. NO: 12, or their respective complementary sequences, and

b) allowing the vector to enter the cells,

wherein said patient has a reduced susceptibility for developing a cancer associated with a mutation in the BRCA2 gene.

15 45. A method according to claim 44 wherein said cells include healthy breast, ovarian or pancreatic tissues.

46. A method according to claim 44 wherein a patient has an inherited mutation in the BRCA2 gene.

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47. A method of treating a patient suspected of having a tumor, comprising:

a) administering to a patient an effective amount of BRCA2 tumor growth inhibitor having an amino acid sequence selected from the group consisting of SEQ. ID. NO: 5, SEQ. ID. NO: 7, SEQ. ID. NO: 9, SEQ. ID. NO: 11, SEQ. ID. NO: 13, any fragments thereto, and any functional equivalent thereof;

b) allowing the patient's cells to take up the protein, and

c) measuring a reduction in tumor growth.

25 48. The method according to claim 47 wherein said tumor is a breast cancer, an ovarian cancer or a pancreatic cancer.

30 49. The method according to claim 47 wherein said patient has an inherited mutation in the BRCA2 gene.

50. A method of preventing the formation or growth of a tumor, comprising:
- 5 a) administering to a patient an effective amount of BRCA2 tumor growth inhibiting protein having an amino acid sequence selected from the group consisting of SEQ. ID. NO: 5, SEQ. ID. NO: 7, SEQ. ID. NO: 9, SEQ. ID. NO: 11, SEQ. ID. NO: 13, any fragments thereto, and any functional equivalent thereof; and
- 10 b) allowing the patient cells to take up the protein.
51. The method according to claim 31 wherein the protein is administered parenternally, by buccal adsorption or inhalation.
52. A cloning vector comprising:
- 10 (a) a DNA sequence as set forth in SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, or any fragments thereof; and
- 15 (b) one or more suitable regulatory sequences to induce replication and/or integration in a host cell.
53. An expression vector comprising a DNA sequence as set forth in SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO: 8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, or any fragments thereof operatively linked to one or more promoter sequences capable of directing expression of said sequence in a host cell.
54. A host cell transformed with the vector according to claim 52 or 53.
- 25 55. A BRCA2 polypeptide which is selected from the group consisting of:
- 20 (a) a fragment of BRCA2 protein sequence as set forth in SEQ. ID. NO: 5, SEQ. ID. NO: 7, SEQ. ID. NO: 9, SEQ. ID. NO: 11, or SEQ. ID. NO: 13;
- 25 (b) an amino acid sequence which is substantially homologous to the BRCA2 protein sequence as set forth in SEQ. ID. NO: 5, SEQ. ID. NO: 7, SEQ. ID. NO: 9, SEQ. ID. NO: 11, or SEQ. ID. NO: 13;
- 30 (c) a molecule which has similar function to the BRCA2 protein; and
- 35 (d) a fusion protein of (a), (b), or (c).

56. An anti-BRCA2 antibody wherein a molecule according to claims 17-21, 23,
25, 27, 29, 31, or 55 is used as an immunogen.
- 5 57. A diagnostic reagent comprising a molecule selected from the group
consisting of:
(a) a DNA sequence as set forth in SEQ. ID. NO: 4, SEQ. ID. NO: 6, SEQ. ID. NO:
8, SEQ. ID. NO: 10, SEQ. ID. NO: 12, or their complementary sequences;
(b) a nucleic acid fragment of (a) comprising at least 10 nucleotide in length;
10 (c) a sequence which hybridizes to (a) or (b);
(d) a polypeptide according to claim 17-21, 23, 25, 27, 29, 31, or 55; and
(e) an antibody which specifically binds to the polypeptide of (d).
- 15 58. A pharmaceutical composition comprising a molecule according to any one of
the claims 17-21, 23, 25, 27, 29, 31, 55 in a pharmaceutically acceptable carrier.
59. A pharmaceutical composition comprising a molecule according to claim 56 in a
pharmaceutically acceptable carrier.
- 20 60. A pharmaceutical composition comprising a molecule according to claim 57 in
a pharmaceutically acceptable carrier.

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